PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

15

Applicant's or agent's file reference HeL/UB 42252	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/SE99/01741	International filing date *(day/month/year)* 01.10.1999	Priority date *(day/month/year)* 01.10.1998
International Patent Classification (IPC) or national classification and IPC7 A 61 K 35/74		
Applicant Probi AB et al		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 6 sheets, including this cover sheet.

☒ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of 1 sheets.

3. This report contains indications relating to the following items:

- I ☒ Basis of the report
- II ☐ Priority
- III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV ☐ Lack of unity of invention
- V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI ☐ Certain documents cited
- VII ☐ Certain defects in the international application
- VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
10.04.2000	29.01.2001
Name and mailing address of the IPEA/SE Patent- och registreringsverket Box 5055 S-102 42 STOCKHOLM Facsimile No. 08-667 72 88	Authorized officer Carolina Palmcrantz/EÖ Telephone No. 08-782 25 00

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Form PCT/IPEA/409 (cover sheet) (January 1998)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/SE99/01741

I. Basis f the report

1. With regard to the **elements** of the international application:*

☐ the international application as originally filed

☒ the description:
pages 1-10 , as originally filed
pages _____ , filed with the demand
pages _____ , filed with the letter of _____

☒ the claims:
pages _____ , as originally filed
pages _____ , as amended (together with any statement) under article 19
pages _____ , filed with the demand
pages 11 , filed with the letter of 14.12.2000

☐ the drawings:
pages _____ , as originally filed
pages _____ , filed with the demand
pages _____ , filed with the letter of _____

☐ the sequence listing part of the description:
pages _____ , as originally filed
pages _____ , filed with the demand
pages _____ . filed with the letter of _____

2. With regard to the **language**. all the elements marked above were available or furnished to this Authority in the language in which the international application was filed. unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language _____ which is:

☐ the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).

☐ the language of publication of the international application (under Rule 48.3(b)).

☐ the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/ or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

☐ contained in the international application in written form.

☐ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☐ furnished subsequently to this Authority in computer readable form.

☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. ☐ The amendments have resulted in the cancellation of:

☐ the description, pages _____

☐ the claims, Nos. _____

☐ the drawings, sheet/fig _____

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2 (c)).**

* *Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).*

** *Any replacement sheet containing such amendments must be referred to under item I and annexed to this report.*

V. Reasoned statement under Article 35(2) with regard t novelty, inventive step r industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	1-10	YES
	Claims		NO
Inventive step (IS)	Claims	1-10	YES
	Claims		NO
Industrial applicability (IA)	Claims	1-10	YES
	Claims		NO

2. Citations and explanations (Rule 70.7)

Amended claims 1-10 were filed 15.12.2000. Claim 1 has been restricted in that the bacterial strain gives rise to a significant increase in the faecal concentration of propionic acid and that the level of oxidative stress factors in the blood are reduced. It is considered that with the wording "significant" is meant faecal propionic acid concentrations above a level which is expected to normally be present, although no levels have been demonstrated in the application.

Thus, the present application pertains to the use of a bacterial strain giving rise to a significant increase in the faecal concentration of propionic acid for the manufacture of a medicament for reduction of the level of oxidative stress factors in blood in mammals including man. The bacterial strain is a strain of Lactobacillus or Propionibacterium, preferably Lactobacillus plantarum 299v, deposition number DSM 9843. The bacterial strain is more specifically used for the prophylaxis and/or treatment of chronic inflammatory diseases exemplified by rheumatic diseases and psoriasis.

The wording "oxidative stress factors" of claim 1 is considered to cover many different compounds, thus making the matter for which protection is sought not clear and concise (c.f. PCT Article 6). This report has therefore been established based upon those parts of the claims that seem to be supported in the application, i.e. for IL-6, reactive oxygen species, and adhesion of monocytes to endothelial cells.

The International Search Report revealed eleven documents:

D1) WO 9426133 A1 (OTSUKA PHARMACEUTICAL CO., LTD.), 24 November 1994 (24.11.94)

.../...

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: V

D2) Dialog Information Service, File 155, Medline, Dialog accession no. 09478714, Medline accession no. 98226319, Nenonen MT et al: "Uncooked, lactobacilli-rich, vegan food and rheumatoid arthritis", Br J Rheumatol (ENGLAND) Mar 1998, 37 (3) p274-81

D3) US 4314995 A (KOSEI HATA ET AL), 9 February 1982 (09.02.82), column 2, line 60 - column 3, line 14; the abstract

D4) File WPI, Derwent accession no. 1993-061603, CALPIS SHOKUHIN KOGYO KK: "Compsns. Contg. Fermented milk or its processed prods. - used to increase produktivity of inerleukin-2 and/or interleukin-3 and/or inhibiting productivity of interleukin 6 and accelerate T- and B cell function"; JP,A,5009124, 19930119, DW199308

D5) Dialog Information Service, File 73, EMBASE, Dialog accession no. 06821350, Embase no. 1997103844, Huang N. et al: "Inhibition of IL-8 gene expression in Caco-2 cells by compounds which induce histone hyperacetylation"; & Cytokine (CYTOKINE) (United Kingdom) 1997, 9/1 (27-36)

D6) Dialog Information Service, File 155, Medline, Dialog accession no. 09429705, Medline accession no. 98142113, Kim YI: "Short-chain fatty acids in ulcerative colitis", Nutr Rev (UNITED STATES) Jan 1998, 56 (1 Pt 1) p17-24

D7) Dialog Information Services, File 5, BIOSIS, Dialog accession no. 10971268, BIOSIS accession no. 199799592413, Siigur U et al: "Effect of bacterial infection and administration of a probiotic on faecal short-chain fatty acids", Microbial Ecology in Health and Disease 9 (6):p271-277 1996

D8) Dialog Information Service, File 5, BIOSIS, Dialog accession no. 10835570, BIOSIS accession no. 199799456715, Lisitsyna T A et al: "Active forms of oxygen and pathogenesis of rheumatic arthritis and systemic lupus erythematosus", Vestnik Rossiiskoi Akademii Meditsinskikh Nauk 0 (12):p15-20 1996

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: V

D9) WO 9800035 A1 (CAVALIERE VED, VESELY), 8 January 1998 (08.01.98)

D10) Dialog Information Services, File 155, MEDLINE, Dialog accession no. 04285102, Medline accession no. 84251869, Nabukhotnyi TK et al: "Use of adapted Maljutka and Malysh Propionibacterium acidophilus mixtures in the combined treatment of acute diseases of the gastrointestinal tract in infants"; 6 Vopr Pitan (USSR) Nov-Dec 1983, (6) p27-30

D11) Dialog Information Services, File 73, EMBASE, Dialog accession no. 07527508, Embase accession no. 1998387565, Tanaka K. et al: "The effects of nonsteroidal anti-inflammatory drugs on immune functions of human peripheral blood monoclear cells"; & Immunopharmacology (IMMUNOPHARMACOLOGY) (Netherlands) 1998, 40/3 (209-217)

D1 concerns an antioxidant food comprising Lactobacillus plantarum. The food is effective in preventing diseases caused by active oxygen.

D2 discloses that an uncooked vegan diet, rich in Lactobacilli, decreased subjective symptoms of rheumatoid arthritis. It is also suggested that large amounts of living lactobacilli consumed daily may have positive effects on rheumatoid arthritis.

D3 concerns pharmaceutical Lactobacillus preparations for the treatment of for instance gastritis and enteritis or other inflammatory diseases before they become chronic.

D4 discloses a composition containing fermented milk. The milk was fermented with lactic acid bactera , e.g. Lactobacillus bulgaricus.The composition inhibit the productivity of interleukin-6 in animal and human cells.

D5 discloses that short chain fatty acids (SCFA), such as propionate, inhibit IL-8 expression and may therefore be effective in the treatment of ulcerative colitis (chronic inflammatory condition of the colon). This is probably due to the inhibition of production of pro-inflammatory substances by the intestinal epithelium.

.../...

Supplemental B x
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: V

None of D1-D5 expressly discusses bacterial strains giving rise to significantly increased faecal concentrations of propionic acid, that is, above a level which is expected to normally be produced. Therefore, claims 1-10 seem to be novel. However, it cannot be excluded that the intended bacteria disclosed in D1-D4, especially Lactobacillus bulgaricus in D4, give rise to significantly increased faecal concentrations of propionic acid. Comparative examples have to be made to show novelty.

Moreover,in view of D1-D5 it is not considered to be obvious to select e.g. Lactobacillus strains, which significantly increases the faecal concentration of propionic acid in order to systemically reduce the level of oxidative stress factors. Therefore, claims 1-10 are considered to fulfil the requirements of inventive step and industrial applicability.

D6-D11 are considered to show the general state of the art and are not considered to be of particular relevance.

CLAIMS

1. Use of a bacterial strain giving rise to a significant increase in the faecal concentration of propionic acid for the manufacture of a medicament for reduction of the level of oxidative stress factors in blood in mammals including man.

2. Use according to claim 1, wherein the bacterial strain is a strain of *Lactobacillus* or *Propionibacterium*.

3. Use according to claim 1 or 2, wherein the bacterial strain is a *Lactobacillus plantarum* strain.

4. Use according to any of claims 1 to 3 of *Lactobacillus plantarum* 299v, deposition number DSM 9843.

5. Use according to any of claims 1-4 for the reduction of IL-6 in blood.

6. Use according to any of claims 1-4 for the reduction of the level of reactive oxygen species in blood.

7. Use according to any of claims 1-4 for the reduction of the adhesion of monocytes to endothelial cells.

8. Use according to any of claims 1 to 4 for the manufacture of a medicament for the prophylaxis and/or treatment of chronic inflammatory diseases.

9. Use according to claim 8, for the prophylaxis and/or treatment of rheumatic diseases.

10. Use according to claim 8, for the prophylaxis and/or treatment of psoriasis.